REMARKS

In this Amendment, claims 3, 32 and 33 are canceled, and claims 34-54 are new. Thus, after entry of this Amendment, claims 34-54 will be pending in the application.

A personal interview was held on November 8, 2005. The required Statement of Substance of Interview was filed on November 9, 2005.

I. SUMMARY OF THE INVENTION

As described in the specification, the present invention relates to an improved fibrinolytic agent for conditions such as deep-vein thrombosis, pulmonary embolism and myocardial infarction.

Streptokinase is a potent plasminogen activator that has been used extensively for the treatment of these circulatory diseases. However, administration of streptokinase leads to the systemic conversion of plasminogen to plasmin, and thus in turn, can result in hemorrhagic complications due to the proteolytic degradation of blood factors by plasmin.

The art has made extensive efforts to engineer clot-specific plasminogen activators to solve these limitations. As stated by Runge et al., *Proc. Natl. Acad. Sci. USA* 88:10337-10341 (1991): "The limitations have prompted generation (by recombinant DNA technology) of hundreds of plasminogen activator mutants, which have produced at best, only modest improvements in thrombolytic therapy."

The present invention provides an improved fibrinolytic agent to solve the limitations in the art. As recited in new claim 34, the fibrinolytic agent is a chimeric polypeptide that comprises a streptokinase component capable of plasminogen activation, and a fibrin-binding component, wherein said fibrin-binding component is fibrin-binding domains 4 and 5 of human

fibronectin, or is fibrin-binding domains 1 and 2 of human fibronectin. The streptokinase component and fibrin-binding component are fused via a peptide bond and are linked through a region that is sufficiently flexible so as to prevent activation of plasminogen by the streptokinase component, and so as to allow plasmin-dependent activation of the streptokinase component.

The chimeric polypeptide is thus engineered to have at least two beneficial properties.

First, the fibrin-binding component adds a clot-specificity to the streptokinase component, thereby delivering the streptokinase component to the site of the clot.

Second, the fibrin-binding component is fused to the streptokinase component through a region having a certain level of flexibility, such that the streptokinase component is initially prevented from activating plasminogen, but is subsequently liberated from the fibrin-binding component(s) by plasmin that will be present, for example, at the site of the clot.

II. SUPPORT FOR CLAIMS 34-53

New claims 34-54 define the present invention as follows.

Independent **claim 34** encompasses the fusion protein with: a fibrin-binding component fused at the N-terminus of the streptokinase component, a fibrin-binding component fused at the C-terminus of streptokinase component, *or* a fibrin-binding component fused at both ends.

New dependent **claims 34-40** are directed to the chimeric polypeptide in which the fibrin-binding component is fused to the N-terminus of the streptokinase component.

New dependent **claims 41-46** are directed to the chimeric polypeptide in which the fibrin-binding component is fused to the C-terminus of the streptokinase component.

New dependent **claims 47-52** are directed to the chimeric polypeptide in which a fibrin-binding component is fused to both the N-terminus and C-terminus of the streptokinase component.

New dependent **claims 53-54** are directed to a pharmaceutical composition comprising the chimeric polypeptide of the invention and a stabilizer.

Claims 34-53 are supported by the specification as follows:

Claim	Exemplary Written Description Support in Original Specification
34. A ***chimeric polypeptide*** comprising the following components:	Page 1, 2nd ¶, lines 1 and 2.
(a) ***a streptokinase component capable of plasminogen activation***; and	Original claim 1.
(b) ***a fibrin-binding component, wherein said fibrin-binding component is fibrin-binding domains 4 and 5 of human fibronectin, or is fibrin-binding domains 1 and 2 of human fibronectin***;	Original claim 1.
wherein said streptokinase component and said fibrin-binding component ***are fused via a peptide bond*** and ***are linked through a region that is sufficiently flexible so as to prevent activation of plasminogen by said streptokinase component***, and	Original claim 1. Page 9, lines 7-19.
so as to allow plasmin-dependent activation of said streptokinase component.	Page 9, lines 15-25.
35. The chimeric polypeptide of claim 34, ***wherein said fibrin-binding component is fused to the N-terminus of said streptokinase component.***	See Fig. 1, construct (c).
36. The chimeric polypeptide of claim 35, wherein said streptokinase component ***is a Streptococcus***	Page 21, 2nd ¶.

equismilis H46A streptokinase component.	
37. The chimeric polypeptide of claim 36, ***wherein the flexible region comprises the N-terminal region of SEQ ID NO: 2.***	Page 17, 3rd ¶; Example 5; Fig. 1, construct (C)
38. The chimeric polypeptide of claim 37, ***wherein said chimeric polypeptide further comprises a transglutaminase cross-linking site.***	Figure 15.
39. The chimeric polypeptide of claim 38, ***wherein said streptokinase component comprises amino acids 1-383 of SEQ ID NO: 2.***	Figure 1, construct (c) (SEQ ID NO: 11); page 37, lines 4-7.
40. The chimeric polypeptide of claim 39, ***wherein said chimeric polypeptide is encoded by the polynucleotide of SEQ ID NO: 11.***	Fig. 21b.
41. The chimeric polypeptide of claim 34, ***wherein said fibrin-binding component is fused to the C-terminus of said streptokinase component.***	See Figure 1, constructs (A) and (B).
42. The chimeric polypeptide of claim 41, ***wherein said streptokinase component is a Streptococcus equismilis H46A streptokinase component.***	Page 21, 2nd ¶.
43. The chimeric polypeptide of claim 42, ***wherein said flexible region is a polypeptide linker comprising Gly-Gly-Gly.***	Figure 15; page 13, 3rd ¶.
44. The chimeric polypeptide of claim 43, ***wherein the flexible region further comprises a transglutaminase crosslinking site.***	Figure 15.

45. The chimeric polypeptide of claim 44, ***wherein said streptokinase component comprises amino acids 1-383 of SEQ ID NO: 2.***	Figure 1, constructs (A) and (B); (SEQ ID NOS 9 and 10); page 37, lines 4-7.
46. The chimeric polypeptide of claim 45, ***wherein said chimeric polypeptide is encoded by the polynucleotide of SEQ ID NO: 9 or SEQ ID NO: 10.***	Figures 17b and 19b.
47. The chimeric polypeptide of claim 34, ***wherein a fibrin-binding component is fused to each of the N-terminal and C-terminal ends of said streptokinase component*** via a peptide bond, and each fibrin-binding component is linked to said streptokinase component through a region that is sufficiently flexible so as to prevent activation of plasminogen by said streptokinase component, and so as to allow plasmin-dependent activation of said streptokinase component.	Figure 1, construct (D)
48. The chimeric polypeptide of claim 47, ***wherein said streptokinase component is a Streptococcus equismilis H46A streptokinase component.***	Page 21, 2nd ¶.
49 The chimeric polypeptide of claim 48, ***wherein the flexible region linking the N-terminus of said streptokinase component to a fibrin-binding component comprises the N-terminal region of SEQ ID NO: 2; and wherein the flexible region linking the C-terminus of said streptokinase component to a fibrin-binding component is a polypeptide linker comprising Gly-Gly-Gly.***	Page 17, 3rd ¶; Example 5; Fig. 1, construct (D) Figure 15; page 13, 3rd ¶.
50. The chimeric polypeptide of claim 49, ***further comprising a transglutaminase crosslinking site.***	Figure 15.
51. The chimeric polypeptide of claim 50, ***wherein said streptokinase component comprises amino acids 1-383 of SEQ ID NO: 2.***	Figure 1, construct (D); (SEQ ID NO: 12); page 37, lines 4-7.

52. The chimeric polypeptide of claim 51, ***wherein said chimeric polypeptide is encoded by the polynucleotide of SEQ ID NO: 12.***	Figure 22B
53. A pharmaceutical composition comprising the chimeric polypeptide of claim 34, ***and a stabilizer.***	Original claim 32.
54. The pharmaceutical composition of claim 53, wherein said stabilizer is ***human serum albumin or mannitol.***	Original claim 32.

No new matter has been introduced.

Entry of this Amendment is respectfully requested.

III. COMPLIANCE WITH SECTION 112, FIRST PARAGRAPH

It is strongly believed that claims 34-54 comply with the written description and enablement requirements, for at least the reasons set forth in this section.

(A) SUMMARY OF THE LAW

The recent Federal Circuit decision (decided August 12, 2005) of *Capon v. Eshhar v. Dudas,* 418 F.3d 1349 (Fed. Cir. 2005) has clarified the role of Section 112 as it pertains to inventions involving nucleic acids and proteins. Specifically, in *Capon,* the Federal Circuit clarified that Section 112 does not require re-analysis in the specification of that which was already known. A copy of this decision is attached to this Amendment for the Examiner's convenience.

A summary of the facts of *Capon* are as follows.

Capon and Eshhar were parties to an interference. The involved claims were directed to chimeric genes. The chimeric genes at issue contained an immunoglobulin gene component and lymphocyte receptor components. The immunoglobulin and receptor gene sequences were each known in the prior art. Neither party's specification provided the nucleotide-by-nucleotide sequences of the genes, nor of a claimed chimeric gene, and as a result, the Board held that neither parties' specification met the written description requirement of 35 U.S.C. §112, first paragraph.

The Patent Office took the position that Federal Circuit precedent required the complete nucleotide-by-nucleotide recitation of chimeric genes in the specification to comply with the written description requirement, and that each parties' claims were broader than the examples provided by the specification, because the claims covered various permutations and combinations of sequences. The Board dissolved the interference and canceled all claims of both parties. Both parties appealed to the Federal Circuit.

Parties Capon and Eshhar argued that the chimeric genes involve selecting and combining ***known*** sequences using ***known*** procedures, and that the law does not require a re-analysis of the nucleotide sequences in order to comply with the written description requirement. The parties further argued that the scope of the claims were fairly described.

In its decision, the Federal Circuit clarified that the description needed to meet the requirements of section 112 ***varies with the nature and scope of the invention at issue***, and that the written description requirement must be applied in the context of the ***particular invention and the state of the knowledge***. The Court distinguished prior written description precedent,

such as *Regents of the University of California v. Eli Lilly & Co.,* 119 F.3d 1559 (Fed. Cir. 1997), as pertaining to DNA that had ***never*** been characterized. The Federal Circuit stated:

> The "written description" requirement must be applied in the context of the particular invention and the state of the knowledge. The Board's rule that the nucleotide sequences of the chimeric genes must be fully presented, although the nucleotide sequences of the component DNA are known, ***is an inappropriate generalization***. When the art includes the nucleotide information, precedent does not set a *per se* rule that the information must be determined afresh.... ***As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution.*** *Id.* at 1358. (emphasis added)

With respect to the scope of the claims, the Federal Circuit clarified

> ***It is not necessary that every permutation within a generally operable invention be effective*** in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention. *Id* at 1359. (emphasis added)
>
> The certainty required of the disclosure ***is not greater than that which is reasonable***, having due regard for the subject matter involved. *Id.* at 1360. (emphasis added)

The Federal Circuit remanded the case for further proceedings to determine whether the full scope of the parties' claims were adequately supported by the corresponding disclosures.

Applicants respectfully request that new claims 34-54 be examined in accordance with the law as clarified by *Capon.*

In addition, the MPEP provides the following examination guidelines with respect to enablement, observance of which is respectfully requested.

(1) "A patent need not teach, and preferably omits, what is well known in the art." MPEP §2164.01 citing *In re Buchner,* 929 F.2d 660 (Fed. Cir. 1991).

(2) "The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue." MPEP §2164.01 citing *In re Angstadt*, 537 F.2d 498 (CCPA 1976).

(3) "[A]n extended period of experimentation may not be undue if the skilled artisan is given sufficient guidance." "The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." MPEP §2164.06 citing *In re Colianni*, 561 F.2d 220 (CCPA 1977) and *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988).

(4) "The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation." MPEP §2164.01.

(5) "If one skilled in the art can readily anticipate the effect of a change within the subject matter to which the claimed invention pertains, then there is predictability in the art." MPEP §2164.03.

(B) SUMMARY OF RELEVANT PRIOR ART

The following information is in the prior art, and should be considered when evaluating the present claims for compliance with Section 112. This information is already of record in this application via the Information Disclosure Statement filed October 25, 2004, and is explained in the declaration of Dr. Sahni filed September 22, 2004.

(1) Wang et al., Crystal structure of the catalytic domain of human plasmin complexed with streptokinase. *Science* 281:1662-5 (1998).

(2) Welfle et al., Conformation and stability of streptokinases from nephritogenic and nonnephritogenic strains of streptococci. *Proteins* 27:26-35 (1997).

(3) Reed et al., Identification of a plasminogen binding region in streptokinase that is necessary for the creation of a functional streptokinase-plasminogen activator complex. *Biochemistry* 34: 10266-71 (1995).

(4) Young et al., Interaction of Streptokinase and Plasminogen. *J. Biol. Chem.* 270: 29601-29606 (1995).

(5) Kim et al., C-terminal peptide of streptokinase, Met369-Pro373, is important in plasminogen activation. *Biochem. Mol. Biol. Int.* (1996).

(6) Lee et al., Identification of the functional importance of valine-19 residue in streptokinase by N-terminal deletion and site-directed mutagenesis. *Biochem. Mol. Biol. Int.* 41:199-207 (1997).

(7) Nihalani et al., Mapping of the plasminogen binding site of streptokinase with short synthetic peptides. *Protein Science* 6:1284-1292 (1997).

(8) Fay et al., Functional analysis of the amino- and carboxyl-termini of streptokinase. *Thromb Haemost.* 79:985-91 (1998).

(9) Young et al., Plasminogen Activation by Streptokinase via a Unique Mechanism. *J. Biol. Chem.* 273:3110-3116 (1998).

(10) Nihalani et al., Role of the amino-terminal region of streptokinase in the generation of a fully functional plasminogen activator complex probed with synthetic peptides. *Protein Science* 7:637-648 (1998).

(11) Cinejero-Lara et al., Analysis of the interactions between streptokinase domains and human plasminogen. *Protein Science* 7:2190-2199 (1998).

(12) Reed et al., A catalytic switch and the conversion of streptokinase to a fibrin-targeted plasminogen activator. *Proc. Natl. Acad. Sci. USA* 96:8879-8883 (1999).

(13) Lee et al., Site-specific alteration of Gly-24 in Streptokinase: its effect on plasminogen activation. *Biochem. Biophys. Res. Comm.* 165:1085-90 (1989).

(14) Lin et al., Mutation of lysines in a plasminogen binding region of streptokinase identifies residues important for generating a functional activator complex. *Biochemistry* 35:16879-85 (1996).

(15) Wu et al., Engineering of Plasmin-Resistant Forms of Streptokinase and Their Production in *Bacillus subtilis*: Streptokinase with Longer Functional Half-Life. *Appl. Environ. Microbiol.* 64:824-829 (1998).

(16) Chaudhary et al., Function of the central domain of streptokinase in substrate plasminogen docking and processing revealed by site-directed mutagenesis. *Protein Science* 8:2791-2805 (1999).

(17) Williams et al., Solution structure of a pair of fibronectin type 1 modules with fibrin binding activity. *J. Mol. Biol.* 235:1302-11 (1994).

(18) Matsuka et al., The NH2-terminal Fibrin-binding Site of Fibronectin is Formed by Interacting Fourth and Fifth Finger Domains. *J. Biol. Chem.* 269: 9539-46 (1994).

(19) Rostagno et al., Further Charcterization of the NH2-terminal Fibrin-binding Site on Fibronectin. *J. Biol. Chem.* 31938-45 (1994).

(20) Rostagno et al., Comparison of the fibrin-binding activities in the N- and C-termini of fibronectin. *Biochem. J.* 338:375-386 (1999).

(21) Potts et al., Solution structure of the N-terminal F1 module pair from human fibronectin. *Biochemistry* 38:8304-12 (1999).

(22) Williams et al., Secondary structure of a pair of fibronectin type 1 modules by two-dimensional nuclear magnetic resonance. *Biochemistry* 32:7388-95 (1993).

It is strongly believed that the content of the prior art is such that the present claims need not recite specific sequences for compliance with Section 112. As in *Capon,* the inventive contribution of the present invention is the linking of known peptide components in such a manner so as to produce a desired property. The individual components are well characterized in the prior art.

(C) EXAMPLES AND GUIDANCE IN THE SPECIFICATION WITH RESPECT TO THE MANNER OF LINKING THE STREPTOKINASE AND FIBRIN-BINDING COMPONENTS

The specification provides the following **working examples**, shown diagrammatically in Figure 1:

(1) fibrin-binding domains 1 and 2 of human fibronectin fused to the C-terminus of a streptokinase component capable of plasminogen activation;

(2) fibrin-binding domains 4 and 5 of human fibronectin fused to the C-terminus of a streptokinase component capable of plasminogen activation;

(3) fibrin-binding domains 4 and 5 of human fibronectin fused to the N-terminus of a streptokinase component capable of plasminogen activation; and

(4) fibrin-binding domains 4 and 5 fused to both the N-terminus and C-terminus of a streptokinase component capable of plasminogen activation.

The streptokinase components capable of plasminogen activation and the fibrin-binding components are fused via flexible regions, such as:

(1) a polypeptide linker comprising Gly-Gly-Gly (see constructs (A), (B) and (D) of Figure 1, Examples 3, 4, and 6);

(2) a polypeptide linker further comprising the transglutaminase crosslinking site Q-A-Q-Q-I(M)-V (see constructs (A), (B), and (D) of Figure 1, Examples 3, 4 and 6); and

(3) the N-terminus of streptokinase (beginning with Ile^{1}) (see constructs (C) and (D) of Figure 1, Examples 5 and 6).

Pages 54-59 of the specification detail the kinetics of plasminogen activation for all constructs of Figure 1, as characterized by an in vitro lag time (see particularly the Table at page 57). The specification demonstrates that the lag period coincides with cleavage of the chimeric polypeptides, an effect that is mediated by trace amounts of plasmin (see page 55). That is, each of the constructs is initially inactive, but is activated in a plasmin-dependent manner.

The Table below summarizes the experimental data set forth in the specification at page 57 with respect to the plasminogen activation kinetics, of constructs (A)-(D) of Figure 1.

Construct	Flexible Region Comprises	Lag Time
(A) SK-FBD(4,5)	**-G-G-G-Q-A-Q-Q-I-V**	10 min.
(B) SK-FBD(1,2)	**-G-G-G-Q-A-Q-Q-M-V**	10.5 min
(C) FBD(4,5)-SK	**-I-A-G-P-Q-W-L-L**	8.0 min
(D) FBD(4,5)-SK-FBD(4,5)	**-I-A-G-P-Q-W-L-L** **-G-G-G-Q-A-Q-Q-I-V**	18 min.

The experimental data show that the invention operates in a predictable manner.

First, the streptokinase and fibrin-binding components can be combined in essentially any order to achieve the initial inactivation followed by similar kinetics of plasmin-dependent activation, which is manifested as a lag time (compare constructs (A)-(D)).

Second, flexibility of the adjoining region is a key factor for predicting the plasmin-dependent activation kinetics of these constructs. More specifically, when FBD(4,5) and FBD(1,2) were individually fused to the C-terminus of streptokinase, using very similar intergenic regions, similar activation kinetics were observed (compare constructs (A) and (B)). The kinetics of plasmin-dependent activation are therapeutically important to minimize systemic conversion of plasminogen to plasmin (see the paragraph bridging pages 9 of the specification).

Third, using the flexible region naturally existing at the N-terminus of streptokinase, and having a very different primary sequence from the linker of constructs (A) and (B), FBD(4,5) fused thereto also gave the desired effect (see construct (C)).

Further still, by fusing FBD(4,5) to SK simultaneously at both ends, using the flexible regions discussed above, the lag time is ***additive***, showing that the flexible region can be used to predictably control the kinetics of plasmin-dependent activation, which is therapeutically important (compare constructs (A), (C) and (D)).

Thus, by linking the two components via a region with sufficient flexibility, as exemplified above, one can obtain and control the plasmin-dependent activation of the claimed chimeric polypeptide.

The specification provides additional **guidance** as to the requisite flexibility of the adjoining region.

For example, page 18 of the specification instructs that appropriate intergenic regions can be composed predominantly of Gly, Ser, Asn, Gln and similar amino acids. Further, incorporating more rigid structures such as beta turns into the intergenic region would provide longer initial lag times. The application also describes steps for the creation of a plasmid having convenient restriction sites in the intergenic region, so that regions with varying flexibility may be conveniently, and routinely, swapped. (See, Figure 15 and description thereof at page 13).

Finally, the Examiner is reminded that the flexibility of a peptide can be reasonably predicted from amino acid composition, and is not dependent on specific primary sequences.

(D) CLAIMS 34-54 COMPLY WITH SECTION 112, FIRST PARAGRAPH

New **claim 34** recites:

> A chimeric polypeptide comprising the following components:
>
> a) a streptokinase component capable of plasminogen activation; and
>
> b) a fibrin-binding component, wherein said fibrin-binding component is fibrin-binding domains 4 and 5 of human fibronectin, or is fibrin-binding domains 1 and 2 of human fibronectin;
>
> wherein said streptokinase component and said fibrin-binding component are fused via a peptide bond and are linked through a region that is sufficiently flexible so as to prevent activation of plasminogen by said streptokinase component, and so as to allow plasmin-dependent activation of said streptokinase component.

In view of the abundance of information available in the prior art with respect to streptokinase and the fibrin-binding domains of human fibronectin, it is respectfully asserted that claim 34 is in compliance with section 112, first paragraph. Such a conclusion is supported by the decision in *Capon v. Esshar v. Dudas* (discussed *supra* at pages 12-19).

Further, given the examples and guidance in the specification, as discussed *supra* at pages 19-22, it is believed that "a region that is sufficiently flexible so as to prevent activation of plasminogen by said streptokinase component, and so as to allow plasmin-dependent activation of said streptokinase component" is sufficiently described and enabled.

The Examiner is reminded that the standard for enablement is not whether experimentation is required, because even extensive experimentation is allowed. The question is whether experimentation is ***undue***. As stated by the Federal Circuit: "The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, ***or if the specification in question provides a reasonable amount of guidance with***

respect to the direction in which the experimentation should proceed." *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988).

Any experimentation that would be required to practice the invention of claim 34 is believed to be routine, in view of the examples and guidance set forth in the specification, as discussed *supra*. In view of the present specification one is equipped with: the guidance that flexibility of the adjoining region is important for obtaining and controlling plasmin-dependent activation; exemplary regions with the appropriate flexibility; guidance as to the appropriate amino acid composition of the adjoining region; and guidance as to the construction of a plasmid so as to aid the routine exchange of intergenic regions to alter the activation kinetics, if so desired.

Claims 36, 42, and 48, further recite *Streptokinase equismilis* H46A streptokinase, the most well-studied streptokinase protein (see page 21, second paragraph, of the specification).

Claims 39, 45, and 51 further recite that the streptokinase component capable of plasminogen activation comprises amino acids 1-383 of SEQ ID NO: 2, as is exemplified in constructs (A)-(D) of Figure 1 (Examples 3-6).

Claims 37, 43, 44, and 49 define the flexible region more narrowly, and as exemplified by constructs (A)-(D) of Figure 1 (Examples 3-6).

Claims 40, 46, and 52 recite specific constructs (A)-(D) of Figure 1 (Examples 3-6).

While the Examiner has the initial burden of establishing that any claim does not comply with section 112, first paragraph, Applicants strongly believe that new claims 34-54 are fully in compliance therewith.

IV. RESPONSE TO SECTION 103 REJECTION

(A) THE SECTION 103 REJECTION

At page 4 of the Office Action, the Examiner rejects claims 3, 32, and 33 under 35 USC §103(a) as being obvious over U.S. Patent 5,434,073 to Dawson et al., in view of Matsuka et al., *J. Biol. Chem.* (1994), and further in view of Goldstein et al., *Thrombosis and Haemostasis* (1996).

Specifically, the Examiner contends that Dawson teaches a fusion protein comprising residues 16-383 of streptokinase for fibrinolytic therapy, and which is activated specifically at the site of a blood clot. The Examiner also contends that Goldstein teaches targeting streptokinase to the site of a blood clot by fusing streptokinase with an anti-fibrin antibody.

The Examiner admits that neither Dawson nor Goldstein teaches the fusion of streptokinase with fibrin-binding domains 1 and 2 or 4 and 5 of human fibronectin.

However, the Examiner contends Matsuka teaches these fibrin-binding domains of human fibronectin.

The Examiner concludes that one of skill in the art would have been motivated to prepare a fusion protein for clot-specific fibrinolytic therapy, by fusing streptokinase, as taught by Dawson and Goldstein, with the fibrin-binding domains taught by Matsuka. The Examiner believes that the art recognized the need for a clot-specific fibrinolytic agent, and that one of skill in the art would have expected the fibrin-binding domains of Matsuka to serve the same targeting function as the anti-fibrin antibody of Goldstein.

(B) SUMMARY OF THE LAW

The Examiner is requested to observe the following laws with respect to the application of 35 USC §103.

(1) The examiner's determination should be based upon the readings of the references to avoid hindsight analysis. "It is difficult but necessary that the decision maker forget what he or she has been taught...about the claimed invention and cast the mind back to the time the invention was made..., ***to occupy the mind of one skilled in the art who is presented only with the references***, and who is normally guided by the then-accepted wisdom in the art." MPEP §2141.01(III) citing *W.L. Gore & Associates, Inc. v. Garlock, Inc,* 721 F.2d 1540 (Fed. Cir. 1983) (emphasis added).

(2) For a *prima facie* case of obviousness, the Examiner must establish that, at the time the present invention was made, one of skill in the art would have had a reasonable expectation of successfully combining the teachings of the references to make the claimed invention. MPEP §2143.02. ***The Examiner must consider all claim limitations in this regard.*** MPEP §2143.03.

(3) ***Obviousness cannot be predicated on what is not known at the time an invention is made***, even if the inherency of a certain feature is later

established. See MPEP §2141.02 citing *In re Rijckaert*, 9 F.2d 1531 (Fed. Cir. 1993).

The invention must be considered as a whole. Disclosed inherent properties are part of "as a whole" inquiry. See MPEP §2141.02.

It is noted that during the personal interview held on November 8, 2005, the Examiners asserted that the plasmin-dependent activation of the present invention is inherent in the claimed polypeptide, and thus, is not relevant to patentability. See Examiner Interview Summary. As should be clear from the above, this is simply incorrect. The Examiner is requested to appropriately consider all limitations of the claims, as is necessary to properly apply section 103.

(C) CLAIMS 34-54 ARE NON-OBVIOUS

Claims 3, 32 and 33 have been canceled, and replaced with claims 34-54.

As the rejection would pertain to new claims 34-54, the present 103 rejection is respectfully traversed because one of skill in the art would not have had a reasonable expectation of successfully making the claimed fusion protein, with the recited properties. As the Examiner knows, a reasonable expectation of success is required for a determination that a claimed invention is *prima facie* obvious, and ***all claim limitations must be considered in this regard.***

More specifically, and as to **claims 34-36, 53 and 54**, the teachings of the prior art do not provide a reasonable expectation of successfully engineering a fusion protein between streptokinase and a fibrin-binding domain of human fibronectin, such that the polypeptide would be initially inactive, but could be subsequently activated by plasmin.

Goldstein is the only reference of record that teaches plasmin-dependent activation of a streptokinase fusion protein. The Examiner is requested to note the title of Goldstein: "A Chimeric Streptokinase with ***Unexpected*** Fibrinolytic Selectivity." Further, by Goldstein's own admission, the plasmin-dependent activation of the molecule disclosed therein was not sufficiently understood such that one of skill in the art could engineer this property in a ***different*** fusion protein on the basis of the teachings therein. For example, Goldstein states at page 438:

> In the present work, the antibody targeting paradigm has also been changed ***but this time by serendipity. In a manner not fully understood, 59D8-SK does not instantly activate plasminogen like SK unless a clot is present and then the activation rate is slower than SK.... With a complete understanding of this phenomenon***, we might design better chimeric SK molecules or perhaps conceive of other ways to render targeted enzymes latent until their activity is needed. (emphasis added).

Further still, Goldstein concludes that the cause of the plasmin-dependent activation ***was due to the presence of the antibody at the N-terminus of streptokinase***, thereby suggesting that the phenomenon would not be expected with any other fusion partner with streptokinase. Specifically, Goldstein states at page 437, right column:

> We tentatively concluded that ***tethering 59D8 to the N-terminus of SK was the primary cause of the lag phase*** of plasminogen activation by 59D8-SK. (emphasis added)

In stark comparison, Malke et al. (U.S. Patent 5,187,098), which is of record in this application, disclose a fusion between streptokinase with yet a different fibrin-binding domain (kringle domains of plasminogen). At column 16, lines 60-63, Malke indicates ***that the resulting***

fusion proteins were active, demonstrating that the plasmin-dependent property of Goldstein is not inherent in the preparation of a fusion protein with streptokinase, and one of skill in the art could not have concluded as such.

Thus, in view of Goldstein (teaching that plasmin-dependent activation of the 59D8-SK fusion protein was "unexpected" a product of serendipity and "not well understood") and Malke et al (teaching that kringle-SK fusions were active), one of skill in the art at the time this invention was made could not have reasonably expected to successfully engineer a fusion protein between streptokinase and fibrin-binding domains of human fibronectin, and having plamin-dependent activation characteristics.

With respect to new **claims 37 and 39**, the prior art does not teach or suggest that the N-terminus of streptokinase (beginning with Ile^1) would naturally contain the requisite flexibility for obtaining plasmin-dependent activation of a streptokinase fusion with a fibrin-binding domain from human fibronectin.

With respect to **claim 40**, the prior art does not teach or suggest that the polypeptide encoded by SEQ ID NO: 11 would have the recited properties of plasmin-dependent activation.

With respect to **claims 41 and 42**, the prior art does not teach or suggest that the fusion of any fibrin-binding domain ***at the C-terminus of streptokinase*** to achieve plasmin-dependent activation. Considering that fusion of a fibrin-binding motif at the N-terminus of streptokinase was "unexpected," serendipitous, and "not well understood" (as indicated by Goldstein), and that Goldstein "tentatively concludes" that the plasmin-dependent properties were due to a fusion of

an antibody at the N-terminus (discussed *supra*), one of skill in the art would certainly have had no expectation of creating this property through a fusion at the ***C-terminus*** of streptokinase.

With respect to **claims 42, 43 and 44**, the prior art does not teach that a polypeptide linker comprising Gly-Gly-Gly and/or a transglutaminase crosslinking site is appropriate for obtaining the requisite flexibility of the adjoining region, so as to produce the plasmin-dependent activation property.

With respect to **claim 46,** the prior art does not teach that the polypeptides encoded by SEQ ID NO: 9 or 10 would have the recited plasmin-dependent characteristics.

With respect to **claims 47 and 48**, the prior art does not teach or suggest that a fibrin-binding domain of human fibronectin can be fused ***to both the N-terminus and C-terminus*** of streptokinase at the same time, and still produce plasmin-dependent activation properties.

With respect to **claims 49, 50 and 51,** the prior art does not teach that the N-terminus of streptokinase (beginning with Ile^1) would naturally contain the requisite flexibility for obtaining plasmin-dependent activation of a streptokinase fusion with a fibrin-binding domain from human fibronectin, and that a polypeptide linker comprising Gly-Gly-Gly and/or a transglutaminase crosslinking site is appropriate for obtaining the requisite flexibility of the adjoining region at the C-terminus of streptokinase, so as to produce the plasmin-dependent activation property.

With respect to **claim 52**, the prior art does not teach that the polypeptide encoded by SEQ ID NO: 12 would have the recited plasmin-dependent characteristics.

In view of the above, withdrawal of this rejection is respectfully requested.

VI. RESPONSE TO SECTION 112 REJECTIONS

The Examiner rejects claims 3 and 32 under 35 USC §112, second paragraph, as being indefinite. Specifically, the Examiner contends that claims 3 and 32 lack antecedent basis for "the time lag ranges between 5 to 30 minutes." The Examiner also contends that claim 33 should recite "*the* genetically engineered hybrid polypeptide plasminogen activator."

At page 3 of the Office Action, the Examiner rejects claims 3, 32 and 33 under 35 USC §112, first paragraph, as containing new matter. The Examiner contends that the limitation "residues 16-383 of SEQ ID NO:2" in independent claim 33 is new matter.

Claims 3, 32 and 33 have been canceled. It is believed that the new claims are not indefinite, and do not introduce new matter as discussed (see Table *infra* at pages 9-12).

VII. RESPONSE TO OBJECTIONS TO CLAIMS AND SPECIFICATION

(1) The Examiner objects to the specification for not referencing the status of the parent application.

The specification has been amended to indicate the status of the parent application.

(2) The Examiner objects to the specification for citing Jackson et al., *Biochemistry* 21:6620 (1982) as teaching that the first 15 and last 31 residues of streptokinase are expendable.

The specification has been amended to correctly cite to Young, K.C. et al., (1995) *J. Biol. Chem.* 270:29601-29606; Jackson KW, Malke H, Ferretti JJ, and Tang J (1986) *Biochemistry* 25:108-114.

(3) The Examiner objects to the amended specification, as set forth in the April 9, 2002 Preliminary Amendment, as containing an error in the reverse primer MY 14.

The Applicants have checked the MY 14 primer for errors, and hereby confirm that the primer is correct as set forth in the Preliminary Amendment.

The Examiner is requested to note that MY14 is a REVERSE primer that hybridizes with the ANTISENSE (3' down stream) sequences of the Fibronectin gene at the end of the FBD 4,5 sequences. Since, by definition, it is homologous with the antisense sequence, it should not be surprising that it will have the "sense" or coding sequence.

(4) The Examiner also objects to the description of the MY-10 primer as containing SK codons 377-383.

As is clear from Figure 3, the MY 10 primer does indeed contain SK codons 377-383. These codons are NOT the ones starting at the 5'-end of the primer (viz. G-TAC-GGA-TCC-) since (as clearly indicated in the description), these nucleotides are appended at the 5'-end so as to incorporate Bam H1 and Bsm I sites into the primer and the PCR product, and thereby facilitate docking of this segment (which after amplification would have FBD(1,2) sequences) back into the SK gene. The Bam H1 site is to facilitate sub-cloning of the amplified FBD segment into an appropriate cloning vector prior to hybrid gene construction. The SK gene codons (AAT-GCT-AGC-TAT-CAT-TTA-GCG-) in the primer are to create a hybrid junction between the SK and FBD sequences interspersed with three Gly codons. The primer, of course, contains the FBD-hybridizing sequences at its 3-prime end to enable it to successfully carry out

initiation of the PCR at the upstream end, while primer MY-6 hybridizes at the downstream end of the FBD(1,2) sequence.

(5) The Examiner objects to the blank space at page 44.

The blank space has been removed.

(6) The Examiner objects to the specification for not disclosing the structure of the fusion proteins used in the experiment shown in Figure 24.

The figure legend has been amended to identify the time curves. The Examiner is requested to note that page 58 of the specification clearly indicates that the data shown in Figure 24 are for SK and SK-FBD (4,5).

(7) The Examiner objects to the claims as not beginning with the phrase "We claim" or "The claims are."

The specification has been amended accordingly.

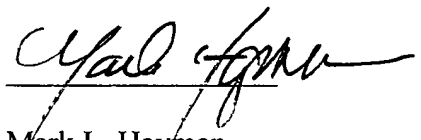
Withdrawal of the objections are respectfully requested.

VIII. CONCLUSION

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge any unpaid fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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